

## T cell reactivity against citrullinated proteins does not differ between healthy persons and patients with inflammatory arthritis

Van Steendam, K. 1, De Ceuleneer, M. 1, De Keyser, F. 2, Elewaut, D. 2, Tilleman, K. 1 and Deforce, D. 1

1 Laboratory for Pharmaceutical Biotechnology, Ghent University, Harelbekestraat 72, B-9000 Ghent, Belgium; 2 Department of Rheumatology, Ghent University Hospital, De Pintelaan 185, B-9000 Ghent, Belgium

### Objectives:

Rheumatoid Arthritis (RA) and Spondyloarthritis (SpA) are two inflammatory auto-immune diseases. They can be distinguished by clinical presentation and the presence of antibodies to citrullinated proteins (ACPA), which is specific for RA. Production of these serological markers indicates a predominant role for B-cells in the pathology of RA. However, little is known about the effect of citrullinated proteins on T-cell reactivity.

### Methods:

PBMC were isolated from healthy volunteers (n=6), RA-patients (CCP+ (n=5) and CCP- (n=5)) and SpA-patients (n=6). The IFN $\gamma$ -production was evaluated by ELISpot analysis. PBMC (500000/100 $\mu$ L) were stimulated with *in vitro* citrullinated (*cit*) and non-citrullinated (*non-cit*) human cell extract, each at a concentration of 200 $\mu$ g/ml and 20U/ml IL2 was added. In order to identify the cells that were crucial in the citrulline-induced T cell reactivity, depletion experiments for CD4, CD8 and HLA-DR were performed according to the manufacturer's protocol (Miltenyi Biotec). In parallel, 10<sup>6</sup> PBMC were cultured for 7 days in the presence of *cit* (80 $\mu$ g/ml) or *non-cit* (80 $\mu$ g/ml). Supernatants were collected and the secretion of cytokines was evaluated by multiple ELISAs (detecting IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, IL-13, IL-17a, IFN $\gamma$ , TNF $\alpha$ , G-CSF and TGF $\beta$ ).

### Results:

In the cultures of PBMCs of healthy persons, the RA and the SpA patients, the number of spot forming counts (SFC) when stimulated with *cit* was higher than when stimulated with *non cit* (significant difference= SFC (cit)- SFC (non cit) > (SD (cit)+ SD (non cit))). A significant difference was seen in 5 of the 6 healthy volunteers and in all the SpA patients. Unexpectedly this phenomenon was less distinct in RA (2/5 CCP+ and 4/5 CCP-), while patients with inflammatory arthritis contain high amounts of citrullinated proteins in their joints.

After depletion of HLA DR positive cells a major drop in reactivity was observed, which illustrates the crucial role of an antigen presenting cell in the citrulline-specific T cell reactivity. Depletion experiments for CD4 and CD8 positive cells showed that both cell types were involved.

The cytokine profile, determined from the multiple ELISAs showed a great resemblance between healthy, RA and SpA patients. For IL2, IL10, IL17 and IL6 an overall greater response to *cit* compared to *non cit* was detected. On the contrary, for TGF $\beta$ , a greater response to *non cit*, compared to *cit*, was seen.

### Conclusion:

These data show that citrulline-reactive IFN $\gamma$  producing T-cells are present in the repertoire of RA patients, SpA patients and healthy people. This implicates that T cell stimulation with citrullinated proteins is a universal mechanism and that ACPA production in RA is not due to the presence of a unique set of citrulline-reactive T cells in the periphery of RA patients.